PROJECT NUMBER: 6908
PROJECT TITLE: Smoke Condensate Studies
PROJECT LEADER: A. H. Warfield
PERIOD COVERED: June, 1989

I. TSNA PRECURSORS

A. **Objective**: To determine the precursors of MS TSNA.

B. **Results**: Data collection was continued as part of an aging experiment on water-washed burley (Bu) filler. The study is nearly complete at this time. After 126 days at 45°C there has been no change in alkaloids, but a ten-fold increase has occurred in endogenous NNK. The samples stored at -30°C were unchanged. MS TSNA determinations have not been completed.

Microencapsulated nicotine bitartrate (µNBT) was added to low alkaloid burley tobacco (LA Bu) as a model for unextracted nicotine. This experiment was designed to determine whether the release of nicotine at temperatures above those at which it would be distilled normally would result in the formation of NNK during smoking. LA Bu (J5BUV) was chosen as a substrate for the experiment because of its high nitrate and low endogenous alkaloid levels, which result in low MS TSNA deliveries with the potential for TSNA pyrosynthesis. MS TSNA levels from the LA Bu with 280°C releasable µNBT were increased by ~40% over control LA Bu. Corresponding increases of ~50-60% occurred when 405°C releasable µNBT was evaluated. However, replication of this experiment is needed to verify these results.

C. **Plans**: The final sample for the washed burley aging study will be analyzed in July, and this study will then be complete. The µNBT experiment will be repeated.

D. **Reference**:

Haut, S. A. Notebook No. 8768, p. 151.

II. UNEXTRACTED NICOTINE STUDIES

A. **Objective**: To determine whether there is a correlation between unextracted nicotine (UN) in base web (BW) and the delivery of NNK in the corresponding MS smoke.

B. **Results**: The initial objective is to measure the levels of total alkali-releasable nicotine (TARN), extractable nicotine (EN), water-soluble UN (Nic-X), and water-insoluble UN (Nic-Y) in three BWs which have a known delivery of MS NNK. The three BWs are BuBW, bright (Br) BW and oriental (Or) BW from the Crossed Solubles Base Web Study. Analysis for nicotine in the extracts is being carried out by HPLC using a cyano column and a methanol-dimethylethanolamine phosphate buffer. TARN is determined by extraction of the BW with 1N NaOH at 90°C for 24 h, and analysis

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of the extract. An extraction with water at 90°C for 24 h removes EN and Nic-X. The marc from the latter extraction contains Nic-Y. Nicotine is measured in this aqueous extract, followed by hydrolysis at 90°C for 24 h, whereupon nicotine is again measured. The difference between the nicotine levels before and after hydrolysis represents Nic-X. Methanol (MeOH) extraction at 22°C for 24 h removes EN, leaving Nic-Y (and possibly Nic-X) in the marc. Preliminary results from this study have shown that BrBW contains more TARN, EN (water), and EN (MeOH) than either BuBW or OrBW. BuBW contains the most Nic-Y, followed by BrBW and OrBW. Extraction with alkali for times longer than 24 h continue to produce more nicotine, and the time will have to be extended to ensure complete removal.

C. **Plans**: More experiments are planned and are now being carried out in order to obtain additional data with the objective of achieving a nicotine balance. Alkali extracted BWs have been submitted to ARD for estimation of nicotine remaining by pyrolysis-MS. Isolation of Nic-X from CEL will be attempted. The water-insoluble marcs will be treated with various enzymes to render Nic-Y soluble enough for isolation. Attempts will be made to identify Nic-X and Nic-Y. Plans will be made to correlate Nic-X and Nic-Y with NNK formation.

D. **References**:

Lambert, E. A. Notebook No. 8523.
Keene, C. K. Notebook No. 8754.

III. ORIENTAL INHIBITOR STUDIES (ORGANIC EXTRACTS)

A. **Objective**: To determine whether the causative agent(s) responsible for the reduced levels of MS TSNA observed for Or tobacco can be removed with organic solvents and applied to other fillers as a means of decreasing the MS TSNA levels delivered by these fillers.

B. **Results**: Four extracts of blended oriental tobacco were prepared by R.Izac to be evaluated for their effect on MS TSNA. The extractions were carried out in a Waring Blender. One batch of tobacco was extracted first with methylene chloride (MeCl) followed by MeOH. A second batch of tobacco was extracted with hexane (HEX) followed by MeCl. The extracts were applied to two experimental fillers: (1) ExBu (Bu tobacco extracted with 5% ethanol/HEX) is depleted in nicotine and endogenous TSNA, but contains minor alkaloids and nitrate and is, therefore, a good substrate for pyrosynthesis; (2) BuBw is low in endogenous TSNA, nicotine, minor alkaloids and nitrate, but produces pyrosynthetic NNK from an unknown precursor. BuBW is a good substrate for evaluating the effect of additives on NNK formation. The results obtained on smoking cigarettes made with the treated ExBu fillers were not conclusive. There were some reductions in MS TSNA relative to the corresponding control, but they were not very large and not reproducible. On a ng/cigarette basis both the HEX and

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MeCl initial extracts gave small reductions which also held true when the data were converted to ng/mg TPM for the most part. None of the secondary extracts gave consistent reductions, but tended to give increases in MS TSNA, due to the presence of considerably higher levels of minor alkaloids found in these extracts than in the initial extracts. When the initial MeCl extract was reevaluated the reductions could not be observed. The HEX extract was not reevaluated. On BuBW, the initial MeCl extract gave a reduction in MS TSNA on a ng/cigarette basis, but this reduction did not hold true when the data were calculated on a ng/mg TPM basis. The initial HEX extract was not evaluated on BuBW.

C. **Plans**: Several samples of Or filler will be extracted by Supercritical Fluid Processing, Inc. in July using supercritical CO_2 under varying conditions to produce extracts that will be tested for inhibition of TSNA pyrosynthesis. Later these extracts will be fractionated in attempts to isolate and identify an inhibitor.

D. **Reference**:

Haut, S. A. Notebook 8768, p. 151.

IV. TSNA IN PROJECT ART MATERIALS

A. **Objective**: To determine the TSNA levels of fillers with lowered nicotine content or other material generated in Project ART studies.

B. **Results**: Three supercritical CO_2 extracted DL5 blend fillers were submitted, along with a control, for MS and filler TSNA analysis. The extractions were carried out by Project 1704 in the 1 L extractor using bright stems, carbon, and a water column as CO_2 scrubbers, respectively, for the three extractions. The filler analyses showed that the use of stems as a scrubber resulted in ~52-62% removal of TSNA, while water and carbon as scrubbers resulted in ~92-97% removal of TSNA. On a ng/cigt. basis, use of the stem scrubber gave a reduction in MS NNN and NAT of 30 and 40%, respectively, while NNK was reduced by 13%. On the same basis, NNN and NNK were reduced by ~55%, while NAT was reduced by ~67% using either water or carbon scrubbers. On a ng/mg TPM basis, stems provided reductions of 11 and 22% for NNN and NAT, respectively, while NNK increased by 14%, which was taken as indicating no change. Water gave a lower TPM value than obtained using carbon as a scrubber, and this caused a differentiation of the results when calculated on a ng/mg TPM basis. Therefore, reductions of 57, 68, and 58%, respectively, were obtained for NNN, NAT, and NNK using carbon as a scrubber, while corresponding reductions of 34, 51 and 33% were obtained using water as a scrubber. A memo discussing this experiment is in preparation.

C. **Reference**:

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Tickle, M. H. Notebook No. 8716.

V. MISCELLANEOUS AND SUPPORT STUDIES

A. **Objective**: To conduct studies of the TSNA content of filler and/or MS smoke or carry out other activities as necessary to support other PM programs.

B. **Results**: A method with improved safety precautions was devised for spraying various solutions onto specific fillers supplied by another group. The sprayings were completed. Tests were conducted on collection methods and analysis for TSNA and VNA in the sidestream chamber. Several problems were encountered, but calibration of the chamber is now being carried out.

C. **References**:

Haut, S. A. Notebook No. 8768, p. 151.
Morgan, W. R. Notebook No. 8579, p. 187.

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